

## TRANSGENIC PATHOGEN-RESISTANT ORGANISM

This is a divisional of application No. 08/457,797, filed on Jun. 1, 1995, now U.S. Pat. No. 5,689,045, which is a continuation of Ser. No. 08/134,416, filed on Oct. 8, 1993, now abandoned.

### FIELD OF THE INVENTION

The invention relates to a pathogen-resistant organism and to a process for generating it.

### BACKGROUND OF THE INVENTION

It is known in the state of the art that infestations of a plant by pathogens causes a series of different reactions. These include, for example, changes in the cell wall structure, the synthesis of phytoalexins which have antimicrobial activity, the accumulation of so-called PR proteins (pathogenesis-related), protease inhibitors and enzymes with hydrolytic functions (Hahlbrock and Grisebach in Ann. Rev. Plant. Physiol., 30 (1979), 105-130).

Many pathogens (fungi and insects) have chitin as a constituent of their cell wall. By contrast, plants possess no chitin. It has now been demonstrated in some cases that there is enhanced production of chitinases in plants after infestation by pathogens. Chitinases are among the enzymes with hydrolytic functions and they catalyze chitin breakdown. It has now been possible to show that plants acquire an increased resistance to pathogens by the production of chitinases.

It is furthermore known to use a gene from barley plants whose gene product codes for an inhibitor of fungal protein synthesis. The incorporation of a corresponding inhibitor gene in transgenic plants led to improved resistance to fungi.

Finally, it has also been disclosed that the use of a polypeptide from *Aspergillus giganteus* is able to protect, by virtue of its antifungal activity, plants from infestation by fungi.

However, given this state of the art there is a need to provide further transgenic pathogen-resistant organisms. Moreover, the organisms which are particularly desired are those whose resistance is increased overall by comparison with the known organisms or is extended with respect to the number of possible pathogens.

This problem is solved by a transgenic pathogen-resistant organism having the features of the present invention.

The invention is based on the surprising finding that the incorporation of at least two different genes with pathogen-inhibiting action into the genome of an organism assists the latter to resist pathogens to an extent going far beyond an additive effect of each of the genes on its own.

The dependent claims indicate further embodiments of the invention.

The genes can code for gene products which reduce the vitality of fungi. In particular, the genes can be of fungal, bacterial and plant, animal or viral origin. In particular, the gene products have properties which promote resistance to fungi. The gene products are chitinase (ChiS, ChiG), glucanase (GluG), protein synthesis inhibitor (PSI) and antifungal protein (AFP).

The transgenic pathogen-resistant organism can be a plant, and tobacco, potato, strawberry, corn, rape or tomato plants are preferred.

The invention also relates to DNA-transfer vectors with inserted DNA sequences as are indicated in detail in this description.

The invention furthermore relates to a process for the generation of pathogen-resistant organisms as are described herein, wherein at least 1 gene with pathogen-inhibiting action is transferred into the genome of an organism, and the pathogen-resistant organism is obtained

(a) by crossing the organism with another, optionally transgenic, organism which contains at least one other gene with pathogen-inhibiting action, and subsequently selecting, and/or

(b) by transformation of this other gene with pathogen-inhibiting action into the organism. The process can be used with DNA-transfer vectors with inserted DNA sequences corresponding to a gene with pathogen-inhibiting action as described herein.

Finally, the invention relates to a process for the generation of pathogen-resistant organisms, wherein vectors which comprise more than one gene with pathogen-inhibiting action are used for the transformation into the genome of an organism.

The invention also relates to a process for ensuring the resistance of organisms to pathogens, characterized in that the organism used is a transgenic pathogen-resistant organism according to the present invention or an organism whose genome contains at least one gene complying with the definitions used herein, and at least one substance which is not expressed by the organism but corresponds to any other one of the gene products complying with the definitions given in this application is applied to the organism.

It was possible to achieve the synergistic effects very particularly with transgenic pathogen-resistant organisms to which the gene sequences which coded for proteins of the attached sequence listings A to E, or corresponded to the latter, were transferred or transfected.

#### ChiS:

A DNA fragment which is 1.8 Kb in size, that codes for a chitinase called ChiS (SEQ ID NO: 8) was isolated from the soil bacterium *Serratia marcescens*. In vitro investigations with purified ChiS protein showed that it is able effectively to inhibit the growth of fungi, even in low concentrations. The reason for the inhibition is that the ChiS protein has a chitinase activity which is able to damage the tips of the fungal hyphae. In this way the fungus is unable to grow further and is inhibited.

#### PSI:

The PSI gene originates from barley and codes for a protein which inhibits protein synthesis by fungi. In vitro tests show that even low concentrations of PSI are sufficient to inhibit various fungi such as, for example, *Rhizoctonia solani*.

#### AFP:

It is possible for a polypeptide which has antifungal activity to be isolated from the fermentation broth of *Aspergillus giganteus* and to be sequenced. This polypeptide is suitable as antifungal agent, for example as spraying agent and as preservative for industrial products and human and animal foods. It can furthermore be combined with other substances which have pesticidal activity, fertilizers or growth regulators. Inhibitory activities against fungi were detectable inter alia against various *Aspergillus*, *Fusaria*, *Phytophthora* and *Trichophyton* species.

#### ChiG and GluG:

Two genes which code, respectively, for a chitinase (ChiG) and glucanase (GluG) can be isolated from certain types of barley. Purified ChiG protein or GluG protein inhibits various phytopathogenic fungi in vitro (inter alia

*Rhizoctonia solani*) (see R. Leah et al., Journal of Biological Chemistry, Vol. 266, No. 3 (1991), pages 1564-1573).

### SUMMARY OF THE INVENTION

The inventors have now found, completely surprisingly, that an at least binary combination of expression of PSI, AFP, ChiS, ChiG or GluG leads to synergistic effects in respect of the acquired resistance to fungi in transgenic plants. In particular, the effects of the individual substances in the combination are markedly exceeded. These include resistance to the fungus *Rhizoctonia solani*, Sclerotinia infestation, Botrytis infestation, etc.

Combinations according to the invention are (DNA and/or polypeptides):

(binary combinations)

ChiS, GluG; ChiS, PSI; ChiS, ChiG; ChiS, AFP; GluG, PSI; GluG, ChiG; GluG, AFP; PSI; ChiG; PSI, AFP; (ternary combinations)

ChiS, GluG, PSI; ChiS, GluG, ChiG; ChiS, GluG, AFP; GluG, PSI, ChiG; GluG, PSI, AFP; PSI, ChiG, AFP; ChiG, AFP, GluG

(quaternary combinations)

ChiS, GluG, PSI, AFP; ChiS, GluG, PSI, ChiG; (quinary combination)

ChiS, GluG, PSI, AFP, ChiG

The invention furthermore relates to the combined use of the proteins with pathogen-inhibiting action, preferably ChiS, PSI, AFP, ChiG and GluG, against pathogens. Combined use also means in this context that at least a first pathogen-inhibiting substance is expressed by the organism and at least a second substance which has pathogen-inhibiting action is applied to the organism from outside.

The agents according to the invention also include those which contain the abovementioned proteins in at least binary combination. The agents according to the invention can contain other active substances besides the proteins. The other active substances can be pesticides, fertilizers and/or growth regulators, and the agents according to the invention can be prepared in various formulations such as concentrates, emulsions, powders, formulations or carriers, mixtures with other active substances, etc. The ChiS/PSI and AFP/PSI combination is particularly preferred. These proteins can be used particularly effectively to inhibit the growth of *Rhizoctonia solani*, especially in tobacco crops.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 shows the effects of AFP and PSI on *Rhizoctonia solani*.

FIG. 2 shows the effects of ChiS and PSI on *Rhizoctonia solani*.

### DETAILED DESCRIPTION OF THE INVENTION

The invention also relates to the use in a process according to the invention of a DNA sequence which codes at least for a polypeptide of sequences A to E, in which sequence A is the sequence of a 60 amino acid AFP protein (SEQ ID NO: 2); sequence A' is the sequence of 51 amino acid AFP protein (SEQ ID NO: 3); sequence B is the sequence of the PSI protein (SEQ ID NO: 5); sequence B' is the sequence of a protein encoded by an incomplete PSI-cDNA clone (SEQ ID NO: 7); sequence D is the sequence of the ChiG protein (SEQ ID NO: 10); and sequence E is the sequence of the GluG protein (SEQ ID NO: 12) or to a pathogen-resistant

organism, where its genome contains at least two different genes under the control of active promoters with pathogen-inhibiting action, where the genes are in each case selected from the group of sequences A to E, in which sequence A is the sequence of a nucleic acid (SEQ ID NO: 1) which comprises a region encoding AFP protein; sequence B is the sequence of a nucleic acid (SEQ ID NO: 4) which comprises a region encoding PSI protein; sequence B' is the sequence of a nucleic acid (SEQ ID NO: 6) which was identified as a portion of an incomplete PSI-cDNA clone; sequence C is the sequence of a nucleic acid (SEQ ID NO: 8) encoding ChiS protein; sequence D is the sequence of a nucleic acid (SEQ ID NO: 9) which comprises a region encoding ChiG protein; and sequence E is the sequence of a nucleic acid (SEQ ID NO: 11) which comprises a region encoding GluG protein. The invention furthermore includes DNA sequences which hybridize with a DNA sequence which codes for polypeptides of amino-acid sequences A to E, in which sequence A is the sequence of a 60 amino acid AFP protein (SEQ ID NO: 2); sequence A' is the sequence of a 51 amino acid AFP protein (SEQ ID NO: 3); sequence B is the sequence of the PSI protein (SEQ ID NO: 5); sequence B' is the sequence of a protein encoded by an incomplete PSI-cDNA clone (SEQ ID NO: 7); sequence D is the sequence of the ChiG protein (SEQ ID NO: 10); and sequence E is the sequence of the GluG protein (SEQ ID NO: 12), where these DNA sequences can be of natural, synthetic or semisynthetic origin and can be related to the abovementioned DNA sequence by mutations, nucleotide substitutions, nucleotide deletions, nucleotide insertions and inversions of nucleotide sequences, and for a polypeptide with pathogenic activity. The invention furthermore relates to a recombinant DNA molecule which contains at least one DNA sequence which accords with the preceding statements, where this DNA molecule can be in the form of a cloning or expression vector.

The invention relates to appropriate host organisms and intermediate hosts which are transformed with a recombinant DNA molecule which accords with the preceding statements. Preferred as intermediate host in the generation of a pathogen-resistant transgenic organism are strains of bacteria, in particular so-called *Agrobacteria* strains.

The invention furthermore relates to the transgenic pathogen-resistant organisms obtained by the process according to the invention, in particular tobacco, potato, corn, pea, rape and tomato plants.

The DNA sequences according to the invention are, as a rule, transferred together with a promoter. Promoter sequences are recognized by the plant transcription apparatus and thus lead to constitutive expression of the gene associated with them in plants. The promoter can, however, also be pathogen-inducible and/or wound-inducible (WUN1) and/or tissue-specific and/or development-specific.

The genetic manipulation operations necessary for carrying out the invention, especially for expression of the gene in plants, are generally known. See for example the publication by Maniatis et al. in "Molecular cloning: A laboratory manual", Cold Spring Harbor (1982).

The invention is explained in detail in the following examples.

All the standard methods of molecular biology were carried out, unless otherwise indicated, as described by Maniatis et al. "Molecular cloning: a laboratory manual", Cold Spring Harbor (1982).

The DNA (SEQ ID NO: 1; SEQ ID NO: 4; SEQ ID NO: 6; SEQ ID NO: 8; SEQ ID NO: 9; SEQ ID NO: 11) coding

for amino-acid sequences A to E (SEQ ID NO: 2; SEQ ID NO: 3; SEQ ID NO: 5; SEQ ID NO: 7; SEQ ID NO: 10; SEQ ID NO: 12) was initially cloned in a manner known per se and then transferred by conjugation into *A. tumefaciens* LBA 4404 (A. Hoekema et al., Nature 303, 179-180). This took place by the method described by Van Haute et al. in EMBO J. 2, 411-418 (1983).

The transfer of DNA into that Agrobacterium was checked by isolating Agrobacterium DNA by the method described by Ebert et al. in Proc. Natl. Acad. Sci. USA 84 5745-5749 (1987). Restriction cleavage of the DNA, transfer to Hybond-N membrane (Amersham) and hybridization with a radioactively labeled DNA probe provided information about successful DNA transfer into the Agrobacterium.

The transformed Agrobacterium was then used to transform tobacco, rape, strawberry, tomato and potato plants.

The LBA4404 Agrobacteria required for the infection were initially cultivated in selective antibiotic medium (P. Zambirsky et al. in EMBO J., 1, 147-152 (1983)), sedimented by centrifugation and washed in YEB medium without antibiotics (YEB=0.5% meat extract; 0.2% yeast extract; 0.5% peptone; 0.5% sucrose; 2 mM MgSO<sub>4</sub>). After renewed sedimentation and taking up in MgSO<sub>4</sub> it was possible to use the bacteria for the infection.

The so-called leaf disk method was used for the infection.

Sterile leaves were used for the leaf disk infection. Leaf pieces about 1 cm in size are dipped in the previously described Agrobacteria suspension and subsequently transferred to 3 MS medium (medium described by T. Murashige and F. Skoog in Physiol. Plant., 15, 473-497 (1962); 3MS=MS-3% sucrose). After incubation at 25° C. to 27° C. with 16 hours of light for two days, the leaf pieces were transferred to MSC16 medium (according to T. Murashige (see above); MSC16=MS+0.5 µg/ml BAP+0.1 µg/ml NAA+100 µg/ml kanamycin sulfate+500 µg/ml Claforan). Shoots appearing after 4-6 weeks were cut off and transplanted to MSC15 medium (according to Murashige (see above); MSC15=MS+2% sucrose, 500 µg/ml Claforan+100 µg/ml kanamycin sulfate). Shoots with root formation were analyzed further.

Monocotyledonous plants (including corn), but some dicotyledonous plants too, were transformed by direct gene transfer into protoplasts. These protoplasts were subsequently regenerated to intact plants (Example: J. Potrykus in Biotechnology 8 (1990), 535).

The resulting transgenic plants were infected with the fungus *Rhizoctonia solani* for testing purposes. For this purpose, fungal cultures were grown and thoroughly mixed in standard soil. This soil was then distributed in a dish and planted with the plants to be tested.

For the evaluation, each plant on a dish was assigned a value from 0 to 3. It was possible to calculate from this for each plant line an index which resulted from the sum of the values. The classification is as follows:

0=no symptoms (healthy)

1=slightly reduced size (compared with a non-infected control); no or very slight visible infestation

2=severe reduction in growth; severe symptoms of infestation

3=dead

The rating is carried out in each case 14 days after the start of the series of tests.

# EXAMPLE 1:

## Fungus inhibition test with combined proteins

The intention initially was to show that the proteins used here have synergistic effects in their combination. Fungal growth tests in vitro were carried out for this purpose.

These entailed a defined amount of *Rhizoctonia solani* fungal mycelium being mixed with 100 µl of potato dextrose solution and incubated in microtiter plates at 25° C. In this test there is a linear correlation between the growth of the fungus and the increase in the optical density at 405 nanometers. The inhibitory effect of proteins can be detected from a smaller increase in the optical density.

2-3 mycelium balls were taken from a liquid culture of *R. solani*, mixed with 100 µl of KGB medium in an Eppendorf vessel and carefully homogenized with a glass mortar. This suspension was then mixed with 10 ml of KGB medium and passed through a sterile 100 µm screen. The optical density of this mycelium fragment suspension (100 µl aliquot) was adjusted to a value of 0.06-0.07 at 405 nanometers by adding medium. 100 µl samples were placed on a microtiter plate and mixed with the proteins to be tested. 7 parallels were measured per mixture. Mixtures which were mixed with the corresponding amounts of buffer served as controls. The plates were incubated in the dark at 25° C. for 48 hours, and the optical density of the cultures was measured at regular intervals.

Calculation of whether two proteins act together in an additive synergistic or antagonistic manner in the inhibition of fungal growth is possible from the measured data with the aid of the Colby formula which is described hereinafter and generally used (S. R. Colby in Wheeds, 15 (1967), 20-22).

To do this it was initially necessary to calculate the growth inhibition E to be expected theoretically with an additive behavior (the expected efficacy). This is given by:

$$E=W1+W2-((W1 \times W2)/100)$$

where W1 and W2 indicate the efficacies of the individual proteins, which is defined as that percentage deviation of the growth plot (in the presence of the protein) from the untreated control. The efficacy for a protein (at a defined time in the growth plot) is given by:

$$W1=(OD(K)-OD(P))/OD(K) \times 100 \text{ (percent)}$$

In this, OD(K) is the optical density of the untreated control and OD(P) is the optical density of the culture treated with the protein.

Thus, on combined use of two proteins, the following statements were possible: if the efficacy G measured in the experiment is identical to the expected value E, the behavior is additive. If, on the other hand, G is greater than E, the behavior is synergistic.

Using this test model, it emerged that the proteins ChiS, PSI, AFP, ChiG and GluG used in the Example surprisingly have synergistic inhibitory effects on various fungi, and these effects were achieved both by the combination of two types of protein and by multiple combination of the above-mentioned proteins.

For example, the following values were determined from the combination of ChiS and PSI protein and from the combination of AFP protein and PSI protein on the fungus *Rhizoctonia solani* (in each case two different ChiS and AFP concentrations with a constant RIP concentration):

ChiS+PSI:

The expected values were: E1=29.9% and E2=44.5%

The measured values were: G1=60.4% and G2=64.1%

The proteins ChiS and PSI therefore act together in a synergistic manner in the inhibition of the growth of *R. solani*.

FIG. 1 shows the results obtained with the combination of the proteins and with the individual substances. According to the Figure, various ChiS concentrations (0.5 µg/ml and 0.05 µg/ml) are combined with PSI protein (1.0 µg/ml).

#### AFP+PSI:

The expected values were: E1=39.9% and E2=41.9%

The measured values were: G1=57.7% and G2=65.4%

The AFP and PSI combination also according to this shows a synergistic inhibition of growth of the fungus *R. solani*. FIG. 2 indicates the test results with various AFP concentrations (0.4 µg/ml and 0.04 µg/ml) combined with PSI protein (1.0 µg/ml).

#### EXAMPLE 2:

##### Transgenic plants

In order to obtain the organisms according to the invention with DNA sequences which act together synergistically, initially transgenic plants which contained at least one of the genes which act together synergistically were generated.

##### ChiS in transgenic slants

Initially a ChiS gene was fused to plant regulatory sequences.

A ChiS gene 1.8 Kb in size was sequenced by using synthetic oligonucleotides in the dideoxy sequencing method of Sanger et al. in Proc. Natl. Acad. Sci. USA, 74 (1977), 5463-5467.

The 35S promoter originating from cauliflower mosaic virus (CamV) (400 bp (according to Töpfer et al. in Nucl. Acid. Res., 15 (1987), 5890)) underwent transcriptional fusion to the ChiS gene. The termination signal, which is 0.2 Kb in size, of the 35S gene of CamV, whose functionality in dicotyledonous plants is known, was used 3' from the ChiS gene. The chimeric gene 35S-ChiS was cloned into the pLS034 vector by means of the *Agrobacterium tumefaciens* transformation system in tobacco and potato plants, and kanamycin-resistant plants were regenerated.

It was possible to detect both the ChiS gene and the corresponding mRNA as well as the gene product protein in the resulting plants.

##### PSI in transgenic plants

PolyA RNA was initially isolated from ripe barley seeds (*Hordeum vulgare* L. cv. Piggy) and deposited in a cDNA gene bank λ-gt-11-phages. The details of the process are to be found in R. Lea in Plant. Biol., 12 (1989), 673-682. Monospecific PSI antibodies were then used to identify cDNA clones.

Subsequently, the PSI-positive λ-gt-11-phages were isolated, cloned further and sequenced by the dideoxy sequencing method of Sanger et al. indicated above. The DNA cloned into *E. coli* was then transferred in the manner described above by conjugation into *Agrobacterium* LBA4404.

Both the transferred gene and mRNA and gene product were detectable in corresponding transgenic tobacco, potato, rape, strawberry and tomato plants.

##### AFP in transgenic plants

For the cloning in the vector, the cDNA sequence of the antifungal peptide is provided with ends which can be ligated into BamHI and SalI restriction cleavage sites. The cloning vector used was pDH51 (Pietrzak et al. in Nucl. Acids Res. 14 (1986), 5857). The vector pDH51 was opened with the restriction enzymes BamHI and SalI between

promoter and terminator. The vector pDH51 is a pUC18 derivative which contains promoter and terminator sequences of the 35S transcript from cauliflower mosaic virus. These sequences are recognized by the plant's transcription apparatus and lead to strong constitutive expression of the gene associated with them in plants. The DNA of the antifungal peptide is then cloned via the BamHI and SalI cleavage site into the vector. Finally, the transcription unit—promoter, gene and terminator—is cut out of the vector using the restriction enzyme EcoRI and cloned into a plant transformation vector. The following vectors and their derivatives can, for example, be used as plant transformation vector:

pOCA18 (Olszewski et al. in Nucl. Acids Res., 16 (1988), 10765) pPCV310 (Koncz and Shell in MGG 204 (1986), 383) and pBin19 (Bevan et al. Nucl. Acids. Res. 12 (1984), 8711)

After the transcription unit and the vector had been ligated via the EcoRI cleavage site, the construct was conjugated into the *Agrobacterium* strain MP90RK (Koncz and Shell (see above)) or IHA101 (Hood et al. in J. Bacteriol. 168 (1986), 1291).

Transgenic tobacco, potato, strawberry, rape and tomato plants were then transformed by the method described above. Transformed shoots are selected on the basis of the cotransferred resistance to the antibiotic kanamycin. Expression of the antifungal protein in the transformed crop plants was checked and confirmed by DNA analysis (Southern blotting), RNA analysis (Northern blotting) and protein analysis with specific antibodies (Western blotting).

##### ChiG and GluG in transgenic plants

ChiG- and GluG-transgenic plants which were both Southern-, Northern- and Western-positive were obtainable in analogy to the plants described above.

ChiS, PSI, AFP, ChiG, GluG in transgenic monocotyledonous plants

It was possible by means of direct gene transfer to integrate the abovementioned genes into the genome of monocotyledonous plants such as, for example, corn. This resulted in transgenic plants which were Southern- and Northern- and Western-positive.

Combination of various fungus-resistance genes in transgenic plants

The previously obtained tobacco, corn, rape, strawberry, potato and tomato plants were crossed together and selected for plants containing in each case the fungus-resistant genes of both parents. In addition, transgenic plants were obtained by transforming them initially with one and then with one or more other gene. Finally, plants were also transformed with vectors which contained various resistance genes. Fungus-resistance tests were done with this plant material. Surprisingly, in all cases synergistic effects, not just additive effects, in respect of fungus resistance are observed.

For example, a tobacco plant which expresses ChiS and PSI shows a considerably greater resistance to *Rhizoctonia* infestation than the plants which expressed only ChiS or PSI or which would result from the additive resistance.

A synergistic inhibitory effect on infestation with *Rhizoctonia solani* also results from combined expression of PSI- and AFP-transgenic tobacco. Combination of two or more different genes (ChiS, RIP, AFP, ChiG and GluG) in a wide variety of transgenic plants also led to synergistic inhibitory effects on various fungi.

Whereas wild-type plants have index values from 38 to 46 in tests on 20 seedlings, it emerges with transgenic tobacco according to the invention that the latter grows as well in the presence of the fungus *Rhizoctonia solani* as do control plants (index value 10-12) cultivated on *Rhizoctonia*-free soil.

## SEQUENCE LISTING

## ( 1 ) GENERAL INFORMATION:

( i i i ) NUMBER OF SEQUENCES: 12

## ( 2 ) INFORMATION FOR SEQ ID NO: 1:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 275 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: cDNA

( v i ) ORIGINAL SOURCE:

- ( A ) ORGANISM: *Aspergillus giganteus*

( i x ) FEATURE:

- ( A ) NAME/KEY: 5'UTR
- ( B ) LOCATION: 1..45

( i x ) FEATURE:

- ( A ) NAME/KEY: CDS
- ( B ) LOCATION: 46..225
- ( C ) IDENTIFICATION METHOD: experimental
- ( D ) OTHER INFORMATION: /codon\_start= 46
  - / function= "antifungal agent"
  - / product= "antifungal peptide"
  - / evidence= EXPERIMENTAL
  - / note= "antifungal agent, especially on  
Rhizoctonia solani, various *Aspergillus*, *Fusaria*  
and *Trichophyton* species"

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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TTGCCACCCC CGTTGAAGCC GATTCTCTCA CCGCTGGTGG TCTGG ATG CAA GAG      54
                                     Met Gln Glu
                                     1
ATG AGA GCG CGG GTT TTG GCC ACA TAC AAT GGC AAA TGC TAC AAG AAG      102
Met Arg Ala Arg Val Leu Ala Thr Tyr Asn Gly Lys Cys Tyr Lys Lys
5          10          15
GAT AAT ATC TGC AAG TAC AAG GCA CAG AGC GGC AAG ACT GCC ATT TGC      150
Asp Asn Ile Cys Lys Tyr Lys Ala Gln Ser Gly Lys Thr Ala Ile Cys
20          25          30          35
AAG TGC TAT GTC AAA AAG TGC CCC CGC GAC GGC GCG AAA TGC GAG TTT      198
Lys Cys Tyr Val Lys Lys Cys Pro Arg Asp Gly Ala Lys Cys Glu Phe
40          45          50
GAC AGC TAC AAG GGG AAG TGC TAC TGC TAGACGGTGA GCGAAGGGAC      245
Asp Ser Tyr Lys Gly Lys Cys Tyr Cys
55          60
GAAGTAGGCT GGGGGTTATT TTACTCTGCT      275

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## ( 2 ) INFORMATION FOR SEQ ID NO: 2:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 60 amino acids
- ( B ) TYPE: amino acid
- ( D ) TOPOLOGY: linear

( i i ) MOLECULE TYPE: protein

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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Met Gln Glu Met Arg Ala Arg Val Leu Ala Thr Tyr Asn Gly Lys Cys      15
1          5          10
Tyr Lys Lys Asp Asn Ile Cys Lys Tyr Lys Ala Gln Ser Gly Lys Thr      30
20          25          30

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Ala Ile Cys Lys Cys Tyr Val Lys Lys Cys Pro Arg Asp Gly Ala Lys  
35 40 45

Cys Glu Phe Asp Ser Tyr Lys Gly Lys Cys Tyr Cys  
50 55 60

( i ) SEQUENCE CHARACTERISTICS:  
( A ) LENGTH: 51 amino acids  
( B ) TYPE: amino acid  
( D ) TOPOLOGY: linear

( v ) FRAGMENT TYPE: C-terminal

( A ) ORGANISM: *Aspergillus giganteus*

( A ) NAME/KEY: Protein  
( B ) LOCATION: 1..51  
( D ) OTHER INFORMATION: /note= "active protein fragment of AFP"

[illegible]

( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 1032 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

( v i ) ORIGINAL SOURCE:  
( A ) ORGANISM: *Hordeum vulgare*  
( B ) STRAIN: L.cv. Piggy

( i x ) FEATURE:  
 ( A ) NAME/KEY: 5'UTR  
 ( B ) LOCATION: 1..42

( i x ) FEATURE:

- ( A ) NAME/KEY: 3'UTR
- ( B ) LOCATION: 886..1032
- ( D ) OTHER INFORMATION: /partial

/ note= "46 nucleotides at the 3'-end not shown."

( i x ) FEATURE:

-continued

(A) NAME/KEY: polyA\_signal  
 (B) LOCATION: 930..935  
 (D) OTHER INFORMATION: /note= "potential polyadenylation  
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## (ix) FEATURE:

(A) NAME/KEY: polyA\_signal  
 (B) LOCATION: 963..976  
 (D) OTHER INFORMATION: /note= "potential polyadenylation  
 signal"

## (ix) FEATURE:

(A) NAME/KEY: polyA\_signal  
 (B) LOCATION: 1002..1011  
 (D) OTHER INFORMATION: /note= "potential polyadenylation  
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## (ix) FEATURE:

(A) NAME/KEY: mat\_peptide  
 (B) LOCATION: 46..886

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

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CTTAATAGCA CATCTTGTCC GTCTTAGCTT TGCATTACAT CC ATG GCG GCA AAG      54
Met Ala Ala Lys
1

ATG GCG AAG AAC GTG GAC AAG CCG CTC TTC ACC GCG ACG TTC AAC GTC      102
Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala Thr Phe Asn Val
5 10 15 20

CAG GCC AGC TCC GCC GAC TAC GCC ACC TTC ATC GCC GGC ATC CGC AAC      150
Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala Gly Ile Arg Asn
25 30 35

AAG CTC CGC AAC CCG GCG CAC TTC TCC CAC AAC CGC CCC GTG CTG CCG      198
Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg Pro Val Leu Pro
40 45 50

CCG GTC GAG CCC AAC GTC CCG CCG AGC AGG TGG TTC CAC GTC GTG CTC      246
Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe His Val Val Leu
55 60 65

AAG GCC TCG CCG ACC AGC GCC GGG CTC ACG CTG GCC ATT CGG GCG GAC      294
Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala Ile Arg Ala Asp
70 75 80

AAC ATC TAC CTG GAG GGC TTC AAG AGC AGC GAC GGC ACC TGG TGG GAG      342
Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly Thr Trp Trp Glu
85 90 95 100

CTC ACC CCG GGC CTC ATC CCC GGC GGC ACC TAC GTC GGG TTC GGC GGC      390
Leu Thr Pro Gly Leu Ile Pro Gly Gly Thr Tyr Val Gly Phe Gly Gly
105 110 115
Ala

ACC TAC CGC GAC CTC CTC GGC GAC ACC GAC AAG CTG ACC AAC GTC GCT      438
Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu Thr Asn Val Ala
120 125 130

CTC GGC CGG CAG CAG CTC CCG GAC GCG GTG ACC GCC CTC CAC GGG CGC      486
Leu Gly Arg Gln Gln Leu Pro Asp Ala Val Thr Ala Leu His Gly Arg
135 140 145
Leu Ala
CTG GCG

ACC AAG GCC GAC AAG CCG TCC GGC CCG AAG CAG CAG CAG GCG AGG GAG      534
Thr Lys Ala Asp Lys Pro Ser Gly Pro Lys Gln Gln Gln Ala Arg Glu
150 155 160

GCG GTG ACG ACG CTG CTC CTC ATG GTG AAC GAG GCC ACG CGG TTC CAG      582
Ala Val Thr Thr Leu Leu Leu Met Val Asn Glu Ala Thr Arg Phe Gln
165 170 175 180

ACG GTG TCT GGG TTC GTG GCC GGG TTG CTG CAC CCC AAG GCG GTG GAG      630
Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro Lys Ala Val Glu
185 190 195

AAG AAG AGC GGG AAG ATC GGC AAT GAG ATG AAG GCC CAG GTG AAC GGG      678
Lys Lys Ser Gly Lys Ile Gly Asn Glu Met Lys Ala Gln Val Asn Gly
200 205 210

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5,804,184

15

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|            |            |            |            |            |            |            |            |             |     |     |     |     |     |     |     |      |
|------------|------------|------------|------------|------------|------------|------------|------------|-------------|-----|-----|-----|-----|-----|-----|-----|------|
| TGG        | CAG        | GAC        | CTG        | TCC        | GCG        | GCG        | CTG        | CTG         | AAG | ACG | GAC | GTG | AAG | CCT | CCG | 726  |
| Trp        | Gln        | Asp        | Leu        | Ser        | Ala        | Ala        | Leu        | Leu         | Lys | Thr | Asp | Val | Lys | Pro | Pro |      |
|            |            | 215        |            |            |            |            | 220        |             |     |     |     | 225 |     |     |     |      |
| CCG        | GGA        | AAG        | TCG        | CCA        | GCG        | AAG        | TTC        | GCG         | CCG | ATC | GAG | AAG | ATG | GGC | GTG | 774  |
| Pro        | Gly        | Lys        | Ser        | Pro        | Ala        | Lys        | Phe        | Ala         | Pro | Ile | Glu | Lys | Met | Gly | Val |      |
|            |            | 230        |            |            |            | 235        |            |             |     |     | 240 |     |     |     |     |      |
| AGG        | ACG        | GCT        | GTA        | CAG        | GCC        | GCC        | AAC        | ACG         | CTG | GGG | ATC | CTG | CTG | TTC | GTG | 822  |
| Arg        | Thr        | Ala        | Val        | Gln        | Ala        | Ala        | Asn        | Thr         | Leu | Gly | Ile | Leu | Leu | Phe | Val |      |
|            |            | 245        |            |            | 250        |            |            |             |     | 255 |     |     |     |     | 260 |      |
| GAG        | GTG        | CCG        | GGT        | GGG        | TTC        | ACG        | GTG        | GCC         | AAG | GCG | CTG | GAG | CTG | TTC | CAT | 870  |
| Glu        | Val        | Pro        | Gly        | Gly        | Leu        | Thr        | Val        | Ala         | Lys | Ala | Leu | Glu | Leu | Phe | His |      |
|            |            |            |            | 265        |            |            |            |             | 270 |     |     |     |     | 275 |     |      |
| GCG        | AGT        | GGT        | GGG        | AAA        | TAGGTAQTTT | TCCAGGTATA | CCTGCATGGG | TAGTGTAATAA |     |     |     |     |     |     |     | 925  |
| Ala        | Ser        | Gly        | Gly        | Lys        |            |            |            |             |     |     |     |     |     |     |     |      |
|            |            |            |            | 280        |            |            |            |             |     |     |     |     |     |     |     |      |
| GTCGAATAAA | CATGTCACAG | AGTGACGGAC | TGATATAAAT | AAATAAATAA | ACGTGTCACA |            |            |             |     |     |     |     |     |     |     | 985  |
| GAGTTACATA | TAAACAAATA | AATAAATAAT | TAAAAATGTC | CAGTTTA    |            |            |            |             |     |     |     |     |     |     |     | 1032 |

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 281 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Met Ala Ala Lys Met Ala Lys Asn Val Asp Lys Pro Leu Phe Thr Ala  
1 5 10 15

Thr Phe Asn Val Gln Ala Ser Ser Ala Asp Tyr Ala Thr Phe Ile Ala  
20 25 30

Gly Ile Arg Asn Lys Leu Arg Asn Pro Ala His Phe Ser His Asn Arg  
35 40 45

Pro Val Leu Pro Pro Val Glu Pro Asn Val Pro Pro Ser Arg Trp Phe  
50 55 60

His Val Val Leu Lys Ala Ser Pro Thr Ser Ala Gly Leu Thr Leu Ala  
65 70 75 80

Ile Arg Ala Asp Asn Ile Tyr Leu Glu Gly Phe Lys Ser Ser Asp Gly  
85 90 95

Thr Trp Trp Glu Leu Thr Pro Gly Leu Ile Pro Gly [Gly] Thr Tyr Val  
100 105 110

Gly Phe Gly Gly Thr Tyr Arg Asp Leu Leu Gly Asp Thr Asp Lys Leu  
115 120 125

Thr Asn Val Ala Leu Gly Arg Gln Gln Leu [Pro] Asp Ala Val Thr Ala  
130 135 140

Leu His Gly Arg Thr Lys Ala Asp Lys Pro Ser Gly Pro Lys Gln Gln  
145 150 155 160

Gln Ala Arg Glu Ala Val Thr Thr Leu Leu Leu Met Val Asn Glu Ala  
165 170 175

Thr Arg Phe Gln Thr Val Ser Gly Phe Val Ala Gly Leu Leu His Pro  
180 185 190

Lys Ala Val Glu Lys Lys Ser Gly Lys Ile Gly Asn Gln Met Lys Ala  
195 200 205

Gln Val Asn Gly Trp Gln Asp Leu Ser Ala Ala Leu Leu Lys Thr Asp  
210 215 220

Val Lys Pro Pro Pro Gly Lys Ser Pro Ala Lys Phe Ala Pro Ile Glu  
225 230 235 240



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Lys Met Gly Val Arg Thr Ala Val Gln Ala Ala Asn Thr Leu Gly Ile  
 245 250 255  
 Leu Leu Phe Val Glu Val Pro Gly Gly Leu Thr Val Ala Lys Ala Leu  
 260 265 270  
 Glu Leu Phe His Ala Ser Gly Gly Lys

## (2) INFORMATION FOR SEQ ID NO: 6:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 480 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: Hordeum vulgare
- (B) STRAIN: Lcv. Piggy

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: cDNA gene bank in lambda-gt-11-phages
- (B) CLONE: incomplete psi cDNA clone

## (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..351
- (D) OTHER INFORMATION: /partial  
 / codon\_start=1  
 / function= "protein synthesis inhibitor"  
 / product= "protein synthesis inhibitor"  
 / standard\_name= "PSI"  
 / note= "aminoterminally incomplete protein from  
 an incomplete PSI cDNA clone"

## (ix) FEATURE:

- (A) NAME/KEY: 3'UTR
- (B) LOCATION: 352..487

## (ix) FEATURE:

- (A) NAME/KEY: polyA\_signal
- (B) LOCATION: 404..409
- (D) OTHER INFORMATION: /note= "potential polyadenylation  
 signal"

## (ix) FEATURE:

- (A) NAME/KEY: polyA\_signal
- (B) LOCATION: 437..442
- (D) OTHER INFORMATION: /note= "potential polyadenylation  
 signal"

## (ix) FEATURE:

- (A) NAME/KEY: polyA\_signal
- (B) LOCATION: 445..450
- (D) OTHER INFORMATION: /note= "potential polyadenylation  
 signal"

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| GCG | GTG | ACG | ACG | CTG | CTC | CTC | ATG | GTG | AAC | GAG | GCC | ACG | CGG | TTC | CAG | 48  |
| Ala | Val | Thr | Thr | Leu | Leu | Leu | Met | Val | Asn | Glu | Ala | Thr | Arg | Phe | Gln |     |
| 1   |     |     |     | 5   |     |     |     | 10  |     |     |     |     |     | 15  |     |     |
| ACG | GTG | TCG | GGG | TTC | GTG | GCC | GGG | CTG | CTG | CAC | CCC | AAG | GCG | GTG | GAG | 96  |
| Thr | Val | Ser | Gly | Phe | Val | Ala | Gly | Leu | His | Pro | Lys | Ala | Val | Glu |     |     |
|     |     |     | 20  |     |     |     | 25  |     |     |     |     | 30  |     |     |     |     |
| AAG | AAG | AGC | GGG | AAG | ATC | GGC | AAT | GAG | ATG | AAG | GCC | CAG | GTG | AAC | GGG | 144 |
| Lys | Lys | Ser | Gly | Lys | Ile | Gly | Asn | Glu | Met | Lys | Ala | Gln | Val | Asn | Gly |     |
|     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |     |     |
| TGG | CAG | GAC | CTG | TCC | GCG | GCG | CTG | CTG | AAG | ACG | GAC | GTG | AAG | CCC | CCG | 192 |
| Trp | Gln | Asp | Leu | Ser | Ala | Ala | Leu | Leu | Lys | Thr | Asp | Val | Lys | Pro | Pro |     |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |     |

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|            |            |            |            |            |             |        |        |      |            |            |     |     |     |     |     |     |
|------------|------------|------------|------------|------------|-------------|--------|--------|------|------------|------------|-----|-----|-----|-----|-----|-----|
| CCG        | GGA        | AAG        | TCG        | CCA        | GCG         | AAG    | TTC    | ACG  | CCG        | ATC        | GAG | AAG | ATG | GGC | GTG | 240 |
| Pro        | Gly        | Lys        | Ser        | Pro        | Ala         | Lys    | Phe    | Thr  | Pro        | Ile        | Glu | Lys | Met | Gly | Val |     |
| 65         |            |            |            |            | 70          |        |        |      | 75         |            |     |     |     | 80  |     |     |
| AGG        | ACT        | GCT        | GAG        | CAG        | GCT         | GCG    | GCT    | ACT  | TTG        | GGG        | ATC | CTG | CTG | TTC | GTT | 288 |
| Arg        | Thr        | Ala        | Glu        | Gln        | Ala         | Ala    | Ala    | Thr  | Leu        | Gly        | Ile | Leu | Leu | Phe | Val |     |
|            |            |            | 85         |            |             |        |        | 90   |            |            |     |     | 95  |     |     |     |
| GAG        | GTG        | CCG        | GGT        | GGG        | TTG         | ACG    | GTG    | GCC  | AAG        | GCG        | CTG | GAG | CTG | TTT | CAT | 336 |
| Glu        | Val        | Pro        | Gly        | Gly        | Leu         | Thr    | Val    | Ala  | Lys        | Ala        | Leu | Glu | Leu | Phe | His |     |
|            |            |            | 100        |            |             |        | 105    |      |            |            |     | 110 |     |     |     |     |
| GCG        | AGT        | GGT        | GGG        | AAA        | TAGG        | TAGTTT | TGCAGG | TATA | CCTGCATGGG | TAAATGTAAA |     |     |     |     |     | 391 |
| Ala        | Ser        | Gly        | Gly        | Lys        |             |        |        |      |            |            |     |     |     |     |     |     |
|            |            |            | 115        |            |             |        |        |      |            |            |     |     |     |     |     |     |
| AGTCGAATAA | AAATGTCACA | GAGTGACGGA | CTGATATAAA | TAAATTAATA | AACATGTCTAT |        |        |      |            |            |     |     |     |     |     | 451 |
| CATGAGTGAC | AGACTGATAT | AAATAAATA  |            |            |             |        |        |      |            |            |     |     |     |     |     | 480 |

## (2) INFORMATION FOR SEQ ID NO: 7:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 117 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: protein

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |  |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|--|
| Ala | Val | Thr | Thr | Leu | Leu | Leu | Met | Val | Asn | Glu | Ala | Thr | Arg | Phe | Gln |  |
| 1   |     |     |     | 5   |     |     |     |     | 10  |     |     |     |     | 15  |     |  |
| Thr | Val | Ser | Gly | Phe | Val | Ala | Gly | Leu | Leu | His | Pro | Lys | Ala | Val | Glu |  |
|     |     |     | 20  |     |     |     |     | 25  |     |     |     |     | 30  |     |     |  |
| Lys | Lys | Ser | Gly | Lys | Ile | Gly | Asn | Glu | Met | Lys | Ala | Gln | Val | Asn | Gly |  |
|     |     |     | 35  |     |     |     | 40  |     |     |     |     | 45  |     |     |     |  |
| Trp | Gln | Asp | Leu | Ser | Ala | Ala | Leu | Leu | Lys | Thr | Asp | Val | Lys | Pro | Pro |  |
|     | 50  |     |     |     |     | 55  |     |     |     |     | 60  |     |     |     |     |  |
| Pro | Gly | Lys | Ser | Pro | Ala | Lys | Phe | Thr | Pro | Ile | Glu | Lys | Met | Gly | Val |  |
| 65  |     |     |     |     | 70  |     |     |     | 75  |     |     |     |     | 80  |     |  |
| Arg | Thr | Ala | Glu | Gln | Ala | Ala | Ala | Thr | Leu | Gly | Ile | Leu | Leu | Phe | Val |  |
|     |     |     | 85  |     |     |     |     | 90  |     |     |     |     | 95  |     |     |  |
| Glu | Val | Pro | Gly | Gly | Leu | Thr | Val | Ala | Lys | Ala | Leu | Glu | Leu | Phe | His |  |
|     |     |     | 100 |     |     |     | 105 |     |     |     |     | 110 |     |     |     |  |
| Ala | Ser | Gly | Gly | Lys |     |     |     |     |     |     |     |     |     |     |     |  |
|     |     |     | 115 |     |     |     |     |     |     |     |     |     |     |     |     |  |

## (2) INFORMATION FOR SEQ ID NO: 8:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 2329 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (ii) MOLECULE TYPE: cDNA

## (vi) ORIGINAL SOURCE:

- (A) ORGANISM: *Serratia marcescens*

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: Cosmid bank from *Serratia marcescens*

## (ix) FEATURE:

- (A) NAME/KEY: misc\_feature
- (B) LOCATION: 1..2329
- (C) IDENTIFICATION METHOD: experimental
- (D) OTHER INFORMATION: /function="exo-chitinase"
- / product= "ChS protein"
- / evidences: EXPERIMENTAL

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/ note= "sequence listing of the ChiS gene from a  
plasmid pLChiS from E.coli A 5187"

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

|  |      |
|--|------|
| CAGGGCGTTG TCAATAATGA CAACACCCTG GCTGAAGAGT GTGGTGCAAT ACTGATAAAT  | 60   |
| ATTTATCTTT CTTAATAGA AAATTCACCTA TCCTTATTG TCATGTTTTT TTTTATTAT    | 120  |
| ATGAAAATAA ATTACGCTT GCTGAATAAA ACCCAGTTGA TAGCGCTCTT GTTTTTGCGC   | 180  |
| CTTTTTTATT TATAGTACTG AATGTACGCG GTGGGAATGA TTATTCGCC ACCTGGAAAG   | 240  |
| ACGCTGTTGT TATTTATGA TTTTAACCTT CGCGGATTAT TCGGGAATTT TTTCTGTTTCG  | 300  |
| GCAATGCATC GCGACGATTA ACTCTTTTAT GTTTATCCTC TCGGAATAAA GGAATCAGTT  | 360  |
| ATGCGCAAAAT TTAATAAACC GCTGTTGGCG CTGTTGATCG GCAGCACGCT GTGTTCCGCG | 420  |
| GCGCAGGCCG CCGCGCCGGG CAAGCCGACC ATCGCCTGGG GCAACACCAA GTTCGCCATC  | 480  |
| GTTGAAGTTG ACCAGGCGGC TACCGCTTAT AATAATTTGG TGAAGGTAAA AAATGCCGCC  | 540  |
| GATGTTTCCG TCTCCTGGA TTTATGGAAT GCGGACACCG GCACGACGGC AAAAGTTTTA   | 600  |
| TTAAATGGCA AAGAGGCGTG GAGTGGTCCT TCAACCGGAT CTTCCGGTAC GGCGAATTTT  | 660  |
| AAAGTGAATA AAGGCGGCCG TTATCAAATG CAGGTGGCAC TGTGCAATGC CGACGGCTGC  | 720  |
| ACCGCCAGTG ACGCCACCGA AATTGTGGTA GCGGACACCG ACGGCAGCCA TTTGGCGCCG  | 780  |
| TTGAAAGAGC CGCTGCTGGA AAAGAATAAA CCGTATAAAC AGAACTCCGG CAAAGTGGTC  | 840  |
| GGTTCTTATT TCGTCGAGTG GGGCGTTTAC GGGCGCAATT TCACCGTCGA CAAGATCCCG  | 900  |
| GCGCAAAACC TGACCCACCT GCTGTACGGC TTTATCCCGA TCTGCGGCGG CAATGGCATC  | 960  |
| AACGACAGCC TGAAGAGAT TGAAGGCAGC TTCCAGGCGT TGCAGCGCTC CTGCCAGGGC   | 1020 |
| CGCGAGGACT TCAAAGTCTC GATCCACGAT CCGTTCGCCC CGCTGCAAAA AGCGCAGAAG  | 1080 |
| GGCGTGACCG CCTGGGATGA CCCCTACAAG GGCAACTTCG GCCAGCTGAT GGCGCTGAAG  | 1140 |
| CAGGCGCATC CTGACCTGAA AATCCTGCCG TCGATCGGCG GCTGGACGCT GTCCGACCCG  | 1200 |
| TTCTTCTTCA TGGGCGACAA GGTGAAGCGC GATCGCTTCG TCGGTTGCGT GAAAGAGTTC  | 1260 |
| CTGCAGACCT GGAAGTTCTT CGACGGCGTG GATATCGACT GGGAGTTCCC GGGCGGCAAA  | 1320 |
| GGCGCCAACC CTAACCTGGG CAGCCCGCAA GACGGGGAAA CCTATGTGCT GCTGATGAAG  | 1380 |
| GAGCTGCGGG CGATGCTGGA TCAGCTGTG GGTGAAACCG GCCGCAAGTA TGAGCTGACC   | 1440 |
| TCCGCCATCA GCGCCGGTAA GGACAAGATC GACAAGGTGG CTTACAACGT TCGCGAGAAC  | 1500 |
| TCGATGGATC ACATCTTCTT GATGAGCTAC GACTTCTATG GCGCCTTCGA TCTGAAGAAC  | 1560 |
| CTGGGGCATC AGACCGCGCT GAATGCGCCG CCCTGGAAAC CGGACACCGC CTACACCACG  | 1620 |
| GTGAACGGCG TCAATGCGCT GCTGGCGCAG GCGCTCAAGC CCGGCAAAAT CGTCGTCGGC  | 1680 |
| ACCGCCATGT ATGGCCGCGG CTGGACCGGG GTGAACGGCT ACCAGAACAA TATTCGTTT   | 1740 |
| ACCGGCACCG CCACCGGGCC GGTAAAGGC ACCTGGGAGA ACGGTATCGT GGAATACCGC   | 1800 |
| CAAATCGCCG GCCAGTTCAT GAGCGGCGAG TGGCAGTATA CCTACGACGC CACGGCGGAA  | 1860 |
| GCGCCTTACG TGTTCAAACC TTCCACCGGC GATCTGATCA CCTTCGACGA TGCCCGCTCG  | 1920 |
| GTGCAGGCTA AAGGCAAGTA CGTGTGGAT AAGCAGCTGG GCGGCTGTT CTCCTGGGAG    | 1980 |
| ATCGACGCGG ATAACGGCGA TATTCTCAAC AGCATGAACG CCAGCCTGGG CAACAGCGCC  | 2040 |
| GGCGTTCAAT AATCGGTTGC AGTGGTTGCC GGGGGATATC CTTTCGCCCC CGGCTTTTTT  | 2100 |
| GCCGACGAAA GTTTTTTTTAC GCCGACAGTA TTGTGGCTCT GCGCCGAGCA AAACGCGCTC | 2160 |
| ATCGGACTCA CCCTTTTGGG TAATCCTTCA GCATTTCTCT CTGCTTTTAA CGGCGATCAC  | 2220 |
| AAAAATAACC GTTCAGATAT TCATCATTCA GCAACAAAGT TTTGGCGTTT TTTAACGGAG  | 2280 |

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TTAAAAACCA GTAAGTTTGT GAGGGTCAGA CCAATGCGCT AAAAATGGG

2 3 2 9

## ( 2 ) INFORMATION FOR SEQ ID NO: 9:

## ( i ) SEQUENCE CHARACTERISTICS:

- ( A ) LENGTH: 1002 base pairs
- ( B ) TYPE: nucleic acid
- ( C ) STRANDEDNESS: single
- ( D ) TOPOLOGY: linear

## ( i i ) MOLECULE TYPE: cDNA

## ( v i ) ORIGINAL SOURCE:

- ( A ) ORGANISM: Hordeum vulgare
- ( B ) STRAIN: L.

## ( i x ) FEATURE:

- ( A ) NAME/KEY: 5'UTR
- ( B ) LOCATION: 1..63

## ( i x ) FEATURE:

- ( A ) NAME/KEY: CDS
- ( B ) LOCATION: 64..861
- ( D ) OTHER INFORMATION: /codon\_start= 64  
/ function= "chitinase"  
/ product= "26 kD preprotein of chitinase G (ChiG)"  
/ note= "antifungal activity, especially on  
Trichoderma reesei and Fusarium sporotrichoides as  
well as Rhizoctonia solani and Botrytis cinerea."

## ( i x ) FEATURE:

- ( A ) NAME/KEY: 3'UTR
- ( B ) LOCATION: 862..1002
- ( D ) OTHER INFORMATION: /partial  
/ note= "11 nucleotides at 3'end not shown"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: polyA\_signal
- ( B ) LOCATION: 905..910
- ( D ) OTHER INFORMATION: /note= "potential polyadenylation  
signal"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: sig\_peptide
- ( B ) LOCATION: 64..294
- ( D ) OTHER INFORMATION: /note= "probable signal peptide  
sequence"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: sig\_peptide
- ( B ) LOCATION: 298..312
- ( D ) OTHER INFORMATION: /note= "probable signal peptide  
sequence"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: sig\_peptide
- ( B ) LOCATION: 349..378
- ( D ) OTHER INFORMATION: /note= "probable signal peptide  
sequence"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: sig\_peptide
- ( B ) LOCATION: 466..588
- ( D ) OTHER INFORMATION: /note= "probable signal peptide  
sequence"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: sig\_peptide
- ( B ) LOCATION: 607..861
- ( D ) OTHER INFORMATION: /note= "probable signal peptide  
sequence"

## ( i x ) FEATURE:

- ( A ) NAME/KEY: mat\_peptide
- ( B ) LOCATION: 133..861

## ( x i ) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

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|  |     |     |     |     |     |     |     |     |     |     |            |            |     |     |     |      |
|--|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|------------|------------|-----|-----|-----|------|
| CCTACGACAG TAGCGTAACG GTAAACACCG AGTACGGTAC TCTGTGCTTT GTTGGCTCGC  |     |     |     |     |     |     |     |     |     |     |            |            |     |     |     | 60   |
| ACA  | ATG | AGA | TCG | CTC | GCG | GTG | GTG | GCC | GTG | GTA | GCC        | ACG        | GTG | GCC |     | 108  |
| Met  | Arg | Ser | Leu | Ala | Val | Val | Val | Ala | Val | Val | Ala        | Thr        | Val | Ala |     |      |
| -23  |     |     | -20 |     |     |     |     | -15 |     |     |            |            | -10 |     |     |      |
| ATG  | GCC | ATC | GGC | ACG | GCG | CGC | GGC | AGC | GTG | TCC | TCC        | ATC        | GTC | TCG | CGC | 156  |
| Met  | Ala | Ile | Gly | Thr | Ala | Arg | Gly | Ser | Val | Ser | Ser        | Ile        | Val | Ser | Arg |      |
|  |     |     | -5  |     |     |     |     | 1   |     |     |            | 5          |     |     |     |      |
| GCA  | CAG | TTT | GAC | CGC | ATG | CTT | CTC | CAC | CGC | AAC | GAC        | GGC        | GCC | TGC | CAG | 204  |
| Ala  | Gln | Phe | Asp | Arg | Met | Leu | Leu | His | Arg | Asn | Asp        | Gly        | Ala | Cys | Gln |      |
|  | 10  |     |     |     |     | 15  |     |     |     |     | 20         |            |     |     |     |      |
| GCC  | AAG | GGC | TTC | TAC | ACC | TAC | GAC | GCC | TTC | GTC | GCC        | GCC        | GCA | GCC | GCC | 252  |
| Ala  | Lys | Gly | Phe | Tyr | Thr | Tyr | Asp | Ala | Phe | Val | Ala        | Ala        | Ala | Ala | Ala |      |
| 25   |     |     |     |     | 30  |     |     |     | 35  |     |            |            |     |     | 40  |      |
| TTC  | CCG | GGC | TTC | GGC | ACC | ACC | GGC | AGC | GCC | GAC | GCC        | CAG        | AAG | CGC | GAG | 300  |
| Phe  | Pro | Gly | Phe | Gly | Thr | Thr | Gly | Ser | Ala | Asp | Ala        | Gln        | Lys | Arg | Gln |      |
|  |     |     |     | 45  |     |     |     |     | 50  |     |            |            |     | 55  |     |      |
| GTG  | GCC | GCC | TTC | CTA | GCA | CAG | ACC | TCC | CAC | GAG | ACC        | ACC        | GGC | GGG | TGG | 348  |
| Val  | Ala | Ala | Phe | Leu | Ala | Gln | Thr | Ser | His | Glu | Thr        | Thr        | Gly | Gly | Trp |      |
|  |     |     | 60  |     |     |     |     | 65  |     |     |            |            | 70  |     |     |      |
| GCG  | ACT | GCA | CCG | GAC | GGG | GCC | TTC | GCC | TGG | GGC | TAC        | TGC        | TTC | AAG | CAG | 396  |
| Ala  | Thr | Ala | Pro | Asp | Gly | Ala | Phe | Ala | Trp | Gly | Tyr        | Cys        | Phe | Lys | Gln |      |
|  |     | 75  |     |     |     | 80  |     |     |     |     |            | 85         |     |     |     |      |
| GAA  | CGT | GGC | GCC | TCC | TCC | GAC | TAC | TGC | ACC | CCG | AGC        | GCA        | CAA | TGG | CCG | 444  |
| Glu  | Arg | Gly | Ala | Ser | Ser | Asp | Tyr | Cys | Thr | Pro | Ser        | Ala        | Gln | Trp | Pro |      |
|  | 90  |     |     |     |     | 95  |     |     |     |     | 100        |            |     |     |     |      |
| TGC  | GCC | CCC | GGG | AAG | CGC | TAC | TAC | GGC | CGC | GGG | CCA        | ATC        | CAG | CTC | TCC | 492  |
| Cys  | Ala | Pro | Gly | Lys | Arg | Tyr | Tyr | Gly | Arg | Gly | Pro        | Ile        | Gln | Leu | Ser |      |
| 105  |     |     |     |     | 110 |     |     |     |     | 115 |            |            |     |     | 120 |      |
| CAC  | AAC | TAC | AAC | TAT | GGA | CCT | GCC | GGC | CGG | GCC | ATC        | GGG        | GTC | GAT | CTG | 540  |
| His  | Asn | Tyr | Asn | Tyr | Gly | Pro | Ala | Gly | Arg | Ala | Ile        | Gly        | Val | Asp | Leu |      |
|  |     |     |     | 125 |     |     |     |     | 130 |     |            |            |     | 135 |     |      |
| CTG  | GCC | AAC | CCG | GAC | CTG | GTG | GCC | ACG | GAC | GCC | ACT        | GTG        | GGC | TTT | AAG | 588  |
| Leu  | Ala | Asn | Pro | Asp | Leu | Val | Ala | Thr | Asp | Ala | Thr        | Val        | Gly | Phe | Lys |      |
|  |     |     | 140 |     |     |     |     | 145 |     |     |            |            | 150 |     |     |      |
| ACG  | GCC | ATC | TGG | TTC | TGG | ATG | ACG | GCG | CAG | CCG | CCC        | AAG        | CCA | TCG | AGC | 636  |
| Thr  | Ala | Ile | Trp | Phe | Trp | Met | Thr | Ala | Gln | Pro | Pro        | Lys        | Pro | Ser | Ser |      |
|  |     |     | 155 |     |     |     | 160 |     |     |     |            | 165        |     |     |     |      |
| CAT  | GCT | GTG | ATC | GCC | GGC | CAG | TGG | AGC | CCG | TCA | GGG        | GCT        | GAC | CGG | GCC | 684  |
| His  | Ala | Val | Ile | Ala | Gly | Gln | Trp | Ser | Pro | Ser | Gly        | Ala        | Asp | Arg | Ala |      |
|  | 170 |     |     |     |     | 175 |     |     |     |     | 180        |            |     |     |     |      |
| GCA  | GGC | CGG | GTG | CCC | GGG | TTT | GGT | GTG | ATC | ACC | AAC        | ATC        | ATC | AAC | GGC | 732  |
| Ala  | Gly | Arg | Val | Pro | Gly | Phe | Gly | Val | Ile | Thr | Asn        | Ile        | Ile | Asn | Gly |      |
| 185  |     |     |     |     |     | 190 |     |     |     | 195 |            |            |     |     | 200 |      |
| GGG  | ATC | GAG | TGC | GGT | CAC | GGG | CAG | GAC | AGC | CGC | GTC        | GCC        | GAT | CGA | ATC | 780  |
| Gly  | Ile | Glu | Cys | Gly | His | Gly | Gln | Asp | Ser | Arg | Val        | Ala        | Asp | Arg | Ile |      |
|  |     |     |     | 205 |     |     |     |     | 210 |     |            |            |     | 215 |     |      |
| GGG  | TTT | TAC | AAG | CGC | TAC | TGT | GAC | ATC | CTC | GGC | GTT        | GGC        | TAC | GGC | AAC | 828  |
| Gly  | Phe | Tyr | Lys | Arg | Tyr | Cys | Asp | Ile | Leu | Gly | Val        | Gly        | Tyr | Gly | Asn |      |
|  |     |     | 220 |     |     |     |     | 225 |     |     |            |            | 230 |     |     |      |
| AAC  | CTC | GAT | TGC | TAC | AGC | CAG | AGA | CCC | TTC | GCC | TAATTAATTA | GTCATGTATT |     |     |     | 881  |
| Asn  | Leu | Asp | Cys | Tyr | Ser | Gln | Arg | Pro | Phe | Ala |            |            |     |     |     |      |
|  |     | 235 |     |     |     |     | 240 |     |     |     |            |            |     |     |     |      |
| AATCTTGGCC CTCCATAAAA TACAATAAGA GCATCGTCTC CTATCTACAT GCTGTAAGAT  |     |     |     |     |     |     |     |     |     |     |            |            |     |     |     | 941  |
| GTAACATATGG TAACCTTTTA TGGGGAACAT AACAAAGGCA TCTCGTATAG ATGCTTTGCT |     |     |     |     |     |     |     |     |     |     |            |            |     |     |     | 1001 |
|  |     |     |     |     |     |     |     |     |     |     |            |            |     |     |     | 1002 |

(2) INFORMATION FOR SEQ ID NO:10:

(1) SEQUENCE CHARACTERISTICS:

-continued

(A) LENGTH: 266 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

```

Met Arg Ser Leu Ala Val Val Val Ala Val Val Ala Thr Val Ala Met
-23      -20      -15      -10
Ala Ile Gly Thr Ala Arg Gly Ser Val Ser Ser Ile Val Ser Arg Ala
      5      1      5
Gln Phe Asp Arg Met Leu Leu His Arg Asn Asp Gly Ala Cys Gln Ala
10      15      20      25
Lys Gly Phe Tyr Thr Tyr Asp Ala Phe Val Ala Ala Ala Ala Ala Phe
      30      35      40
Pro Gly Phe Gly Thr Thr Gly Ser Ala Asp Ala Gln Lys Arg Glu Val
      45      50      55
Ala Ala Phe Leu Ala Gln Thr Ser His Glu Thr Thr Gly Gly Trp Ala
      60      65      70
Thr Ala Pro Asp Gly Ala Phe Ala Trp Gly Tyr Cys Phe Lys Gln Gln
      75      80      85
Arg Gly Ala Ser Ser Asp Tyr Cys Thr Pro Ser Ala Gln Trp Pro Cys
90      95      100      105
Ala Pro Gly Lys Arg Tyr Tyr Gly Arg Gly Pro Ile Gln Leu Ser His
      110      115      120
Asn Tyr Asn Tyr Gly Pro Ala Gly Arg Ala Ile Gly Val Asp Leu Leu
      125      130      135
Ala Asn Pro Asp Leu Val Ala Thr Asp Ala Thr Val Gly Phe Lys Thr
      140      145      150
Ala Ile Trp Phe Trp Met Thr Ala Gln Pro Pro Lys Pro Ser Ser His
      155      160      165
Ala Val Ile Ala Gly Gln Trp Ser Pro Ser Gly Ala Asp Arg Ala Ala
170      175      180      185
Gly Arg Val Pro Gly Phe Gly Val Ile Thr Asn Ile Ile Asn Gly Gly
      190      195      200
Ile Glu Cys Gly His Gly Gln Asp Ser Arg Val Ala Asp Arg Ile Gly
      205      210      215
Phe Tyr Lys Arg Tyr Cys Asp Ile Leu Gly Val Gly Tyr Gly Asn Asn
      220      225      230
Leu Asp Cys Tyr Ser Gln Arg Pro Phe Ala
      235      240

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(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 1235 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: *Hordeum vulgare*  
 (B) STRAIN: L

(ix) FEATURE:

(A) NAME/KEY: 5'UTR  
 (B) LOCATION: 1..48

(ix) FEATURE:

(A) NAME/KEY: CDS

-continued

( B ) LOCATION: 49..1050  
 ( D ) OTHER INFORMATION: /partial  
     / codon\_start= 49  
     / function= "glucanase"  
     / product= "preprotein of the glucanase GluG"

( i x ) FEATURE:  
 ( A ) NAME/KEY: 3'UTR  
 ( B ) LOCATION: 1051..1235  
 ( D ) OTHER INFORMATION: /partial  
     / note= "14 nucleotides at the 3'end not shown."

( i x ) FEATURE:  
 ( A ) NAME/KEY: polyA\_signal  
 ( B ) LOCATION: 1083..1088  
 ( D ) OTHER INFORMATION: /note= "potential polyadenylation  
     signal"

( i x ) FEATURE:  
 ( A ) NAME/KEY: polyA\_signal  
 ( B ) LOCATION: 1210..1215  
 ( D ) OTHER INFORMATION: /note= "potential polyadenylation  
     signal"

( i x ) FEATURE:  
 ( A ) NAME/KEY: mat\_peptide  
 ( B ) LOCATION: 133..1050

( x i ) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

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GGCAGCATTG CATAGCATTT GAGCACCAGA TACTCCGTGT GTGCACCA ATG GCT AGA      57
Met Ala Arg
-28

AAA GAT GTT GCC TCC ATG TTT GCA GTT GCT CTC TTC ATT GGA GCA TTC      105
Lys Asp Val Ala Ser Met Phe Ala Val Ala Leu Phe Ile Gly Ala Phe
-25 -20 -15 -10

GCT GCT GTT CCT ACG AGT GTG CAG TCC ATC GGC GTA TGC TAC GGC GTG      153
Ala Ala Val Pro Thr Ser Val Gln Ser Ile Gly Val Cys Tyr Gly Val
-5 1 5

ATC GGC AAC AAC CTC CCC TCC CGG AGC GAC GTG GTG CAG CTC TAC AGG      201
Ile Gly Asn Asn Leu Pro Ser Arg Ser Asp Val Val Gln Leu Tyr Arg
10 15 20

TCC AAG GGC ATC AAC GGC ATG CGC ATC TAC TTC GCC GAC GGG CAG GCC      249
Ser Lys Gly Ile Asn Gly Met Arg Ile Tyr Phe Ala Asp Gly Gln Ala
25 30 35

CTC TCG GCC GTC CGC AAC TCC GGC ATC GGC CTC ATC CTC GAC ATC GGC      297
Leu Ser Ala Val Arg Asn Ser Gly Ile Gly Leu Ile Leu Asp Ile Gly
40 45 50 55

AAC GAC CAG CTC GCC AAC ATC GCC GCC AGC ACC TCC AAC GCG GCC TCC      345
Asn Asp Gln Leu Ala Asn Ile Ala Ala Ser Thr Ser Asn Ala Ala Ser
60 65 70

TGG GTC CAG AAC AAC GTG CGG CCC TAC TAC CCT GCC GTG AAC ATC AAG      393
Trp Val Gln Asn Asn Val Arg Pro Tyr Tyr Pro Ala Val Asn Ile Lys
75 80 85

TAC ATC GCC GCC GGC AAC GAG GTG CAG GGC GGC GCC ACG CAG AGC ATC      441
Tyr Ile Ala Ala Gly Asn Glu Val Gln Gly Gly Ala Thr Gln Ser Ile
90 95 100

CTG CCG GCC ATG CGC AAC CTC AAC GCG GCC CTC TCC GCG GCG GGG CTC      489
Leu Pro Ala Met Arg Asn Leu Asn Ala Ala Leu Ser Ala Ala Gly Leu
105 110 115

GGC GCC ATC AAG GTG TCC ACC TCC ATC CGG TTC GAC GAG GTG GCC AAC      537
Gly Ala Ile Lys Val Ser Thr Ser Ile Arg Phe Asp Gln Val Ala Asn
120 125 130 135

TCC TTC CCG CCC TCC GCC GGC GTG TTC AAG AAC GCC TAC ATG ACG GAC      585
Ser Phe Pro Pro Ser Ala Gly Val Phe Lys Asn Ala Tyr Met Thr Asp
140 145 150

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5,804,184

31

32

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|            |            |            |            |            |            |     |     |     |     |     |            |            |     |     |     |      |
|------------|------------|------------|------------|------------|------------|-----|-----|-----|-----|-----|------------|------------|-----|-----|-----|------|
| GTG        | GCC        | CGG        | CTC        | CTG        | GCG        | AGC | ACC | GGC | GCG | CCG | CTG        | CTC        | GCC | AAC | GTC | 633  |
| Val        | Ala        | Arg        | Leu        | Leu        | Ala        | Ser | Thr | Gly | Ala | Pro | Leu        | Leu        | Ala | Asn | Val |      |
|            |            | 155        |            |            |            |     |     | 160 |     |     |            |            | 165 |     |     |      |
| TAC        | CCC        | TAC        | TTC        | GCG        | TAC        | CGT | GAC | AAC | CCC | GGG | AGC        | ATC        | AGC | CTG | AAC | 681  |
| Tyr        | Pro        | Tyr        | Phe        | Ala        | Tyr        | Arg | Asp | Asn | Pro | Gly | Ser        | Ile        | Ser | Leu | Asn |      |
|            |            | 170        |            |            |            |     | 175 |     |     |     |            | 180        |     |     |     |      |
| TAC        | GCG        | ACG        | TTC        | CAG        | CCG        | GGC | ACC | ACC | GTG | CGT | GAC        | CAG        | AAC | AAC | GGG | 729  |
| Tyr        | Ala        | Thr        | Phe        | Gln        | Pro        | Gly | Thr | Thr | Val | Arg | Asp        | Gln        | Asn | Asn | Gly |      |
|            | 185        |            |            |            |            | 190 |     |     |     |     | 195        |            |     |     |     |      |
| CTG        | ACC        | TAC        | ACG        | TCC        | CTG        | TTC | GAC | GCG | ATG | GTG | GAC        | GCC        | GTG | TAC | GCG | 777  |
| Leu        | Thr        | Tyr        | Thr        | Ser        | Leu        | Phe | Asp | Ala | Met | Val | Asp        | Ala        | Val | Tyr | Ala |      |
|            | 200        |            |            |            | 205        |     |     |     | 210 |     |            |            |     |     | 215 |      |
| GCG        | CTG        | GAG        | AAG        | GCC        | GGC        | GCG | CCG | GCG | GTG | AAG | GTG        | GTG        | GTG | TCG | GAG | 825  |
| Ala        | Leu        | Glu        | Lys        | Ala        | Gly        | Ala | Pro | Ala | Val | Lys | Val        | Val        | Val | Ser | Glu |      |
|            |            |            |            | 220        |            |     |     |     | 225 |     |            |            |     |     | 230 |      |
| AGC        | GGG        | TGG        | CCG        | TCG        | GCG        | GGC | GGG | TTT | GCG | GCG | TCG        | GCC        | GGC | AAT | GCG | 873  |
| Ser        | Gly        | Trp        | Pro        | Ser        | Ala        | Gly | Gly | Ile | Phe | Ala | Ala        | Ser        | Ala | Gly | Asn |      |
|            |            |            | 235        |            |            |     |     | 240 |     |     |            |            | 245 |     |     |      |
| CGG        | ACG        | TAC        | AAC        | CAG        | GGG        | CTG | ATC | AAC | CAC | GTC | GGC        | GGG        | GGC | ACG | CCC | 921  |
| Arg        | Thr        | Tyr        | Asn        | Gln        | Gly        | Leu | Ile | Asn | His | Val | Gly        | Gly        | Gly | Thr | Pro |      |
|            |            | 250        |            |            |            |     | 255 |     |     |     |            | 260        |     |     |     |      |
| AAG        | AAG        | CGG        | GAG        | GCG        | CTG        | GAG | ACG | TAC | ATC | TTC | GCC        | ATG        | TTC | AAC | GAG | 969  |
| Lys        | Lys        | Arg        | Glu        | Ala        | Leu        | Glu | Thr | Tyr | Ile | Phe | Ala        | Met        | Phe | Asn | Glu |      |
|            | 265        |            |            |            |            | 270 |     |     |     |     | 275        |            |     |     |     |      |
| AAC        | CAG        | AAG        | ACC        | GGG        | GAC        | GCC | ACG | GAG | AGG | AGC | TTC        | GGG        | CTC | TTC | AAC | 1017 |
| Asn        | Gln        | Lys        | Thr        | Gly        | Asp        | Ala | Thr | Glu | Arg | Ser | Phe        | Gly        | Leu | Phe | Asn |      |
|            | 280        |            |            |            | 285        |     |     |     | 290 |     |            |            |     |     | 295 |      |
| CCG        | GAC        | AAG        | TCG        | CCG        | GCA        | TAC | AAC | ATC | CAG | TTC | TAGTACGTGT | AGCTACCTAG |     |     |     | 1070 |
| Pro        | Asp        | Lys        | Ser        | Pro        | Ala        | Tyr | Asn | Ile | Gln | Phe |            |            |     |     |     |      |
|            |            |            |            | 300        |            |     |     |     | 305 |     |            |            |     |     |     |      |
| CTCACATACC | TAAATAAATA | AGCTGCACGT | ACGTACGTAA | TGCGGCATCC | AAGTGTAACG |     |     |     |     |     |            |            |     |     |     | 1130 |
| TAGACACGTA | CATTCATCCA | TGGAAGAGTG | CAACCAAGCA | TGCGTTAACT | TCCTGGTGAT |     |     |     |     |     |            |            |     |     |     | 1190 |
| GATACATCAT | CATGGTATGA | ATAAAAGATA | TGGAAGATGT | TATGA      |            |     |     |     |     |     |            |            |     |     |     | 1235 |

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 334 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Met Ala Arg Lys Asp Val Ala Ser Met Phe Ala Val Ala Leu Phe Ile  
 -28 -25 -20 -15

Gly Ala Phe Ala Ala Val Pro Thr Ser Val Gln Ser Ile Gly Val Cys  
 -10 -5 1

Tyr Gly Val Ile Gly Asn Asn Leu Pro Ser Arg Ser Asp Val Val Gln  
 5 10 15 20

Leu Tyr Arg Ser Lys Gly Ile Asn Gly Met Arg Ile Tyr Phe Ala Asp  
 25 30 35

Gly Gln Ala Leu Ser Ala Val Arg Asn Ser Gly Ile Gly Leu Ile Leu  
 40 45 50

Asp Ile Gly Asn Asp Gln Leu Ala Asn Ile Ala Ala Ser Thr Ser Asn  
 55 60 65

Ala Ala Ser Trp Val Gln Asn Asn Val Arg Pro Tyr Tyr Pro Ala Val  
 70 75 80